Abstract title: Assessment of the oxidative stress levels in culture media as a biomarker of embryo cohort quality.

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Study question:
Are the oxidative stress levels measured in grouped cultured embryos spent culture media a biomarker of culture conditions, media quality or embryo quality?

Summary answer:
Optimum culture conditions linked with better pregnancy outcomes were related to oxidative stress levels.

What is known already:
Embryo's in vitro environment is known to affect their development and subsequent clinical outcome. The establishment of optimum culture conditions is therefore crucial aiming at the standardization of in vitro fertilization (IVF) protocols. The Thermochemiluminescence (TCL) Analyzer™ (Carmel Diagnostics, Israel) determines the oxidative status of the embryo's spent culture media assessing the adequacy of the embryo culture conditions, in terms of media, humidity, gas concentration, temperature, etc. The aim of the present study is to assess the use of a novel quality control device in IVF laboratories.

Study design, size, duration:
A prospective cohort study on 471 spent embryo culture media collected from 312 IVF cycles that were analysed from May 2017 to December 2018. Embryos were group cultured and monitored with time-lapse incubators: Embryoscope+® (Vitrolife) and Geri® (Genea). 15 µl/dish of embryo culture media were analysed through TCL Analyzer (Carmel Diagnosis).

Participants/materials, setting, methods:
The TCL Analyzer™ working mechanism consists on the heat-induced oxidation of biological fluids, leading to the production of light energy counted as photons emitted per second (cps). Cps amplitude is assessed in a 300-second period, where TCL parameters are obtained from: cps after 55 seconds (H1), 155 seconds (H2) and 255 seconds (H3). A smoothing algorithm (sm) was used to normalize data. Our results were statistically analysed by a multifactor Anova test.

Main results and the role of chance:
Even though both incubators allow cultivating 16 embryos in group, significantly higher oxidative stress was found in samples collected from Geri dishes than from ESD+ ones (p<.05). This might be due to the amount of culture medium in each dish (80µl and 180µl, respectively) and the impact over the concentration of free radicals. Samples from embryo cohorts cultured since blastocyst stage in single-step culture media (s-s) showed higher TCL parameters than those from embryos cultured in sequential culture media: H1sm (cps)= 93.24 for two-step vs. 98.75 for s-s, H2sm (cps)= 96.51 for two-step vs. 102.57 for s-s and H3sm (cps)= 104.89 for two-step vs 111.09 for s-s. In addition, samples provided from egg donor programs had higher oxidative stress values than those provided from cycles with autologous eggs. The mean and standard deviation of the TCL parameters were as follows (cps): 96.82 ± 39.34 for autologous eggs vs. 103.62±41.58 for donated eggs. Additionally, culture media from embryo cohorts that lead at least one ongoing pregnancy had significantly higher TCL parameters (p<.05). Culture media of embryo cohorts with no pregnancy showed an average of 92.08 ± 42.08 cps and 108.02 ± 39.88 cps for those with one or more pregnancy.
Limitations, reasons for caution:
There is a need to further assess the wide number of different independent variables in culture conditions that may be affecting the oxidative stress levels. Number of embryos cultured in each dish can vary depending on patient and may potentially affect the oxidation.

Wider implications of the findings:
TCL Analyzer™'s assessment has proven relationship with culture conditions (type of incubator and type of media) embryo quality and reproductive outcome. Those values with higher oxidation were related with superior embryo quality and higher chances of a successful pregnancy.

Trial registration number:
Not applicable

Keywords:
Oxidative stress
embryo cohort
group embryo culture